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THE INFLUENCE OF THE TANNIN CONTENT OF THE HOST PLANT ON ENDOTHIA PARASITICA AND RELATED SPECIES

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THE INFLUENCE OF THE TANNIN CONTENT OF THE HOST PLANT ON ENDOTHIA PARASITICA AND RELATED SPECIES.*

By
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and
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FOREWORD.

The very great destructiveness of the chestnut bark blight disease (*Endothia parasitica*) and the interesting problems presented by its introduction and spread, made it desirable that it should be studied from every possible angle. Among other interesting phases of the subject was the one here presented, *i. e.*, "The relationship of *Endothia parasitica* and related species to the tannin content of the host plant." The work was done, in co-operation, by the Office of Investigations in Forest Pathology, of the Bureau of Plant Industry of the United States Department of Agriculture, the New

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Jersey Agricultural Experiment Station, and Mr. George A. Kerr, chemist of Lynchburg, Virginia, a collaborator of the Bureau of Plant Industry. The junior author, special agent for the United States Department of Agriculture, was stationed at the New Jersey Agricultural Experiment Station from July 1st, 1913, to July 1st, 1914, working with the senior author, Plant Pathologist of the New Jersey Station. Cultures were furnished by Dr. Haven Metcalf, Dr. F. D. Heald, Dr. C. L. Shear and Dr. Niel H. Stevens of the United States Department of Agriculture; Dr. George P. Clinton of the Connecticut Agricultural Experiment Station; Dr. P. J. Anderson of the Pennsylvania Chestnut Tree Blight Commission, and others. The bark extracts were prepared and chemical analyses made by Mr. George A. Kerr, of Lynchburg, Virginia.

I.

INTRODUCTION.

The resistance of individuals, races and species to certain fungi causing diseases to which their near relatives are subject, and the restrictions of organisms parasitic on plants to more or less definite geographical ranges are problems for which we have little more than theoretical explanations. These problems necessitate a thorough knowledge of both host and parasite and involve a knowledge of morphology, chemistry, physiology and pathology; and it will no doubt require many years of research by many workers before we find satisfactory solutions.

Some years ago the senior author undertook the study of the relation of parasitic fungi to the cell contents of the host plants. Dr. J. J. Taubenhaus was later associated in this work.[1] Their attention was given almost entirely to tannin because of its great abundance in plants. They endeavored to determine to what extent it might be a factor enabling the host plant to resist the attacks of parasitic fungi. The major part of their work was with fungi which attack fruits but, among other species used in this work, was *Endothia parasitica* with which they obtained a good growth of mycelium and a few spores on 0.6 per cent. tannin medium, the

[1] Cook, Mel. T., and Taubenhaus, J. J.—The Relation of Parasitic Fungi to the Cell Contents of the Host Plants (I. The Toxicity of Tannin). Delaware Agricultural Experiment Station, Bul. 91, pp. 1–77, Figs. 1–43; 1911.

highest used with this species. They concluded that "this organism will tolerate large amounts of tannin if the food supply is suitable for its growth" (p. 21). In liquid media the organism showed a lesser tolerance to tannin (p. 29) than on agar. In the summary of their results, they raise the question as to "whether the toxic action was due to the tannin or to the acid radicle" of the completed culture medium. From their work with this and other fungi they conclude that, without regard to the effect of the acid radicle on the medium, tannin itself is toxic to many fungi (p. 30). Their work with *E. parasitica* was very limited, and since this organism grows in the bark, a part of the tree containing a high percentage of tannin, it appeared desirable that the work should be continued.

A little later, Clinton[1] made studies on the relationship of *Endothia* to tannin, using cultures of *E. gyrosa* from oak and *E. parasitica* from the chestnut on potato agar containing tannic acid (M. C. E. brand U. S. P.) in amounts varying from 0.2 to 14 per cent. In this work all cultures grew in media in which less than 4 per cent. tannic acid was used. With 8 per cent. tannic acid about one-half the cultures of *Endothia gyrosa* were eliminated and there was no growth of this organism in cultures containing more than 12 per cent. *Endothia parasitica* was more vigorous; about one-half the cultures grew on 11 per cent. of tannic acid, one culture out of 32 grew on 14 per cent. From his work Clinton concludes that the tannic acid is first oxidized and later in part disappears, to the amount of about one-half the acidity of the completed culture medium. This was determined by titration.

Small quantities (0.2 to 0.8 per cent.) of tannic acid stimulate growth of mycelium and conidial formation in both species of fungi. *E. gyrosa* is less resistant to tannic acid than is *E. parasitica*. The bad effects of tannin were shown by change of color of the mycelium and by modified habit of growth. The two species showed quite marked differences in habit and color on the lower percentages of tannic acid, while on the higher percentages these points of difference disappear. As both the brand of tannic acid used and the medium into which it was placed are different from those used in our own experiments, the points of difference between the results obtained by Clinton and those detailed in the present paper may

[1] Clinton, G. P.—Chestnut Bark Disease, *Endothia gyrosa* var. *parasitica* (Murr.) Clint. Ann. Report Conn. State Agr. Exp. Sta., 1912; pp. 359–453, pl. 21–28, 1913.

be more apparent than real. However, these results are not absolutely comparable to our own on account of these differences in technique, while the results obtained by Cook and Taubenhaus are comparable, as both the media and the commercial tannic acid used by them were similar to those used in the experiments recorded in our work.

The fact that tannin was toxic to the germination of spores and growth of mycelium of many fungi led various observers to the belief that the presence of a higher percentage of tannin in the southern than in the northern chestnut might serve to check, or possibly to prevent, the ravages of the disease in the southern states. However, the report of the Chemist of the Pennsylvania Chestnut Disease Tree Blight Commission indicated that tannin was more abundant in diseased than in healthy bark. This report says:

"In twenty tests, all but one showed a higher percentage of tannin in the infected bark. The lower percentage in the exceptional case was explained as follows. The canker was a very old one. The bark was much cracked and broken. These cracks and breaks evidently permitted rain to leach out both tannin and other soluble matter. But in the remainder of the cases it is a matter of absorbing interest to note an excessive amount of tannin in the infected bark. No satisfactory explanation has been offered as to this tannin increment."[1]

"The next question of interest was the nature of the soluble solids or non-tannins from the infected area. The extracts from the normal bark gave a bright claret colored solution, while that from infected areas was always of a dark brown color. Detanning an extract from normal bark left a solution of a straw yellow color. Detanning the extract from infected bark removes but little color from the solution. Further, in extracting bark from sound and infected areas with cold water, there is produced, in the infected extract only, a heavy muddy sedimentation which responds to tests for gallic acid."[2]

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[1] Mr. George A. Kerr, technological chemist of Lynchburg, Virginia, who was associated with the authors in this work, has made investigations on this point and says: "The increment of tannin is only apparent and does not really occur. We have found all decayed wood and bark give higher tannin contents, no matter what causes the decay. It simply means that other constituents have decomposed and disappeared while the tannin remains practically stable."

[2] Mr. Kerr's investigations indicate that these characteristics are common to decayed wood and bark without regard to the causes.

"The reducing power on Fehling's solution was tried to further investigate the nature of the non tan solution. In every case but one the reducing sugar was much greater in the solution from infected bark. The exceptional case here was the same one noticed previously."

We are unable to find anything in the Report of the Chemist of the Pennsylvania Chestnut Blight Commission that enables us to determine the effects of *Endothia parasitica* on tannin either in the bark or in extracts.

Meanwhile, the progress of the disease demonstrated that the presence of normal quantities of tannin or other toxic substances in living trees could not prevent the spread of the fungus. The problem of the relative importance of tannin in the host plants remained unsolved and led to the undertaking of the studies recorded in this paper. The difficulties in the way of pursuing work of this character and drawing any satisfactory conclusions can be appreciated only by those who have had experience in the study of tannin.

It is plainly evident to the writers that commercial tannin is a very uncertain substance, as packages of tannin from the same manufacturers and supposed to be the same were found to give different results when used in cultures. It was also evident that ordinary methods of determining tannin are unsatisfactory. Therefore, Mr. George A. Kerr, a well known technological chemist of Lynchburg, Virginia, who has devoted considerable attention to the study of tannin, was asked to co-operate in this work. He furnished the writers with the following extracts which he describes as follows:

"No. 1-X is the water soluble tannin from the chestnut bark. It is not soluble in alcohol nor most of the other solvents of like nature. Its reactions with metallic salts, however, are almost identical with sample 2-X which is soluble in both alcohol and water.

"The bark contents of No. 1-X vary from 3 per cent. to 5 per cent. and, to make a culture under conditions approximating the bark contents, I would recommend that a quantity be used not to exceed 5 per cent. of the dry weight of the culture media. This sample is between 95 per cent. and 100 per cent. pure.

"No. 2-X is similar to 1-X except that it is also soluble in alcohol. It is of equal purity. It is probably a glucoside.

"No. 3-X is the coloring matter of the bark, which, under all ordinary methods of analysis, is estimated as tannin, but whether it is true tannin or not I do not know. It precipitates gelatine and combines with hide, but it does not give the distinct reaction with metallic salts that the other two tannins do. The sample is between 85 per cent. and 90 per cent. pure."

Compound A is composed of—

60% tannin, embodying all the tannins referred to above.
10% fermentable sugars.
7% gallic acid.
8% Pentoses and Pentosans.
5% water.
10% undetermined.

The above extracts, and commercial tannins were used in this work as will be hereafter seen. (Pages 24 to 35.)

SOURCE OF CULTURES.

Cultures of various American species of *Endothia,* as well as foreign strains of some of these species, were obtained from various laboratories, as indicated on page 4, and throughout the paper. In addition some strains of *E. parasitica* were isolated in our own laboratory. In the course of our studies various strains of these fungi were used. We have indicated these by the names and the serial numbers used by the laboratories when they came to us, except in the case of *E. parasitica.* In this instance we have uniformly referred to this fungus as *E. parasitica* without regard to whether it was considered a species or a subspecies at the source of supply.

The use of the specific names of *E. gyrosa* and *E. radicalis* varies in different laboratories according to a paper by Shear and Stevens.[1] In the light of this paper it appears that the fungi from Dr. Clinton labelled *E. gyrosa* and that from Dr. Stevens called *E. radicalis* are in reality identical. Our culture work also leads us to the same conclusion. Therefore, in order to avoid confusion we shall use the name *E. radicalis* but shall in each case indicate the origin of our original culture.

[1] Shear, C. L., and Stevens, N. E.—Cultural characters of the chestnut blight fungus and its near relatives. U. S. Dept. Agr., Bur. Plant Indus.; Cir. 131, pp. 1–18, 1913.

A careful study of our cultures indicates that we have but three distinct species: *Endothia parasitica* (American and Chinese strains), *E. radicalis* (*E. gyrosa* and *E. virginiana*) and *E. radicalis mississippiensis.*

II.

PRELIMINARY WORK.

Cultures on Liquid Media.

On account of the anticipated difficulties with tannin in an agar medium, a number of experiments were tried in the hope of finding a satisfactory liquid culture medium in which the nitrogenous elements would be present in forms other than proteid. The results obtained with these media showed a wide range of variation, some proving quite satisfactory while others even failed to germinate the spores of the fungi with which they were inoculated.

In this series of experiments the following formula (referred to as No. II) was taken as the basis of the tests:

Water	1000.00 c.c.
Glucose	20.00 gm.
Peptone	10.00 gm.
Dipotassium phosphate	0.25 gm.
Magnesium sulphate	0.25 gm.

This medium was finally modified by using monobasic potassium instead of dibasic potassium. (See page 12.)

A given series of cultures was always made from the same lot of medium, treated with the same extract, inoculated in the same manner and kept under exactly the same conditions. This rule was adhered to throughout the entire series of investigations.

The above formula was tested with *Endothia radicalis* (European strain, *E. gyrosa* Clinton *E. virginiana*) and *E. parasitica.* All made good growths, filling the tubes or flasks in the course of two or three weeks and producing abundant pycnospores. In these cultures the pycnospores first germinated in contact with the glass, and from this, a perypherial mycelial mat was formed which sent out hyphae until the entire receptacle was filled. Free floating colonies were rarely formed. Pycnidial cushions were usually formed first on the walls of the flask, just above the surface of the

liquid, but later often appeared on the surface of the floating mycelium.

The second formula tried (referred to as No. XII) was as follows:

Water	1000 c.c.
Glucose	20 gm.
Ammonium nitrate	1 gm.
Potassium nitrate	1 gm.
Ammonium sulphate	1 gm.
Magnesium sulphate	0.25 gm.
Dipotassium phosphate	0.25 gm.
Calcium chloride	0.01 gm.

The same fungi were used, but only a fair growth obtained. In this medium the growth, instead of following the sides of the container, formed free swimming globular masses, which in a flask containing 50 c.c. of culture medium grew to be about 1-3 cm. in diameter. These mycelial masses were more or less granulated in appearance. No pycnospores were ever found on these cultures.

A third formula tried (referred to as No. XIII) is as follows:

Water	1000 c.c.
Glucose	10 gm.
Dipotassium phosphate	1 gm.
Magnesium sulphate	0.2 gm.

On this medium the germination took place on the sides of the glass as in the first series, but failed to grow further than to produce a pronounced membrane. This soon died and pulled free from the glass.

As the last two of these media were unpromising, further experiments were undertaken with the first of the above formulas (No. II) as the basis. First, a series of experiments to determine what modifications could be made of this formula without impairing the growth of the fungi. Both monobasic and dibasic potassium phosphate were tried. The effect of omitting the phosphate entirely was also tested. These three series of cultures showed that there was very little choice between the two forms of phosphate, while a fair growth was obtained in the entire absence of phosphate. Tests were next made with these three modified formulas to determine the effect of omitting the peptone. Fair growth was obtained when phosphate was used, but its absence made the medium worthless. Between the two forms of potassium phosphate there was again very slight choice.

Attention was now turned to finding a substitute for the peptone in the culture medium, as this was supposed to be absolutely incompatible with the introduction into the medium of a high percentage of tannin. In the first series of experiments the peptone of the first of the above formulas was replaced by various nitrogenous substances in quantities varying from ¼ to 1 gm. per liter. As might have been expected, this series of cultures proved highly unsatisfactory. We then had the nitrogen equivalents computed for a number of substances and prepared culture media on this basis. First, all peptone was omitted with nothing to replace it. This proved very unsatisfactory as the fungi grew but little past the germination stage. Potassium nitrite was then tried, but proved completely toxic to the various species of *Endothia, Cladosporium* and *Nectria* sown on it. No spores germinated in any of these cultures while checks in other media gave good growth. The only successful inoculation in this medium was with a species of *Sphaeropsis* from Staphyllea which made an abundant mycelial growth but failed to produce spores. Urea was also tried, but at the end of a month the fungus had produced scarcely as much mycelium as had grown on the unmodified control medium in four or five days.

Asparagin was next tried as a substitute for peptone, its nitrogen equivalent being such as to require 7 gm. per liter. This gave a good steady normal growth in all the species of *Endothia* tested. At the end of about four weeks an abundance of conidia were produced. The mycelium was healthy in color and appearance, but did not grow so rapidly as on the unmodified check medium. This medium was in every way satisfactory except that the less vigorous growth of the fungus made it less suitable for our cultures. Indeed, this medium was quite promising, especially as no antagonism to tannin was shown. However, it was abandoned in favor of a solid medium which was later found to be quite satisfactory. (Page 12.)

In the preceding studies certain peculiarities of growth in liquid media were noted which were not comparable to growths in solid media and in the chestnut bark. An effort was made to overcome this difficulty by placing gypsum plugs in the tubes, hoping that they might serve as artificial substrata. These were unsatisfactory because the part above the liquid did not absorb suffi-

cient moisture to induce the desired growth of mycelium and because the part below the liquid absorbed and retained various substances, thus making them unavailable for the fungus.

Relation of Tannin to Culture Medium.

Tests were made with the various constituents of the culture medium (No. II) to be used to determine the relationship of these substances to tannin. It was found that in a medium containing proteid and dibasic potassium phosphate, the latter forms an insoluble precipitate with tannin. This difficulty was eliminated by using monobasic potassium phosphate, which our experiments show gave equally satisfactory results in the growth of the fungi used in our studies. Therefore, Medium No. II (page 9) with monobasic potassium instead of dibasic potassium was used in all future experiments.

The difficulties arising from the use of tannin in a medium containing proteid was not so easily overcome. The first difficulty encountered is the fact that commercial tannin (Merck) is an unstable and variable substance. According to Fischer (Der Deutsch Chem. Ges., 36:3252, 1913) tannin is an anhydrous glucoside of gallic acid. This relationship makes it easily convertible by hydrolosis into gallic acid and related substances. *It is therefore entirely possible that no sterile culture medium can be prepared which contains all the tannin unchanged.*

The usual statement that tannin in contact with proteid forms an insoluble precipitate has not been borne out by our work. Indeed, comparatively large quantities of tannin may be added to the agar formula which we used without changing perceptibly either the tannin or the proteid in so far at least as we were able to determine.

The experiments were conducted with two lots of Merck's tannin. The first of these was already in stock at the time the work was undertaken. By using a 10 per cent. aqueous solution of this tannin, as much as 2 per cent. of tannic acid could be added to the agar without changing the composition of either. However, to accomplish this it was necessary to allow the tannin solution to run slowly from a pipette into the melted agar while the latter was constantly agitated. If the tannin was added too rapidly, or the agitation of the agar was insufficient, more or less coagulation re-

sulted. With a 20 per cent. aqueous solution of tannic acid less than half this amount (0.8 per cent.) could be added without coagulation. Moreover, even a very small amount of tannic acid in its solid form would cause coagulation in the agar. About six months later a second lot of Merck's tannin was secured. Of this only about half as much tannic acid could be added to the agar without change, as of the first lot.

When first placed in the agar, the tannic acid caused a milky appearance which disappeared upon sterilization. Where high percentages of tannin were used, the agar, upon sterilization, showed tendencies of becoming viscid (about 0.8 per cent. to 2.0 per cent.) or even liquid (about 2.0 per cent. to 2.5 per cent.). The transition between viscosity and liquefaction is gradual in such a series as we used, where each member differed from the next by 0.2 per cent. of tannic acid. In no case was the distinct curd which various investigators have described to be observed. In agar with 3.0 per cent. of tannic acid the entire mass of medium becomes a clear liquid with a thin film of solid matter on one side of the test tube if it is set for a slant, or in the bottom if set upright. This solid material gives the same reaction both to Millon's proteid test and to the ammonium molybdate test for tannic acid as does the solid agar of the lower members of the series. Similar results were obtained by testing the liquid portion of the medium. Evidently, the explanation of this liquefaction is to be found in some other direction than the chemical interaction of tannin and proteid.

If the agar medium used is titrated to various degrees of acidity and a series of such tubes sterilized, it is found that the agar ranges from solid through viscid to liquid. That is, the same phenomenon can be induced by acidulating the medium as by the addition of tannic acid. In each case a more careful test of the nature of the proteid substances in the liquid from the acidulated agar shows that proteid digestion has progressed so far that the power of solidification has been lost.

These considerations naturally raise the question of the acidity of the culture medium containing tannic acid. Our tests showed that a 3 per cent. aqueous solution of tannic acid is about +65, Fuller's scale. This is considerably higher than is indicated by Clinton (Rept. Connecticut Exp. Sta. 1912, p. 432). However,

as Clinton used a vegetable (potato agar) medium, while we used a synthetic medium (No. II), the results are in nowise comparable as no account is taken by Clinton of the effects on the tannic acid of the various organic constituents of the medium to which he added it.

Gallic acid was similarly tested, but failed to show any coagulating effect either on the agar or its constituents.

The various materials furnished by Kerr behave in much the same way towards agar as does commercial tannin. His purer tannin extracts, however, do not liquefy the agar at as low percentages as does commercial tannin. Those extracts from which the coloring matter had not been removed had a more pronounced action on the culture medium (page 7) than even the second lot of Merck's tannin. The original acidity of the agar and the quantity and nature of the impurities which may be present in the tannin appear to modify to a great extent the chemical activities upon the admixture of the two substances in completing the culture medium.

Relation of the Fungus to Ether.

According to our first information, certain tannin extracts to be used in the course of the work were to be supplied in solution in ether. It was accordingly deemed expedient to determine the behavior of the fungi under observation when subjected to ether. The method adopted necessitated the use of a liquid medium (page 9) in test tubes which, in addition to the usual cotton plugs, were supplied with close fitting cork stoppers to retard as much as possible the escape of the ether.

In the first series of cultures quantitative work was not attempted, as the primary object was to determine whether or not the fungus would grow in ether. Approximately 10 c.c. of medium was placed in each tube and one, two or three drops of ether added from a pipette (*i. e.,* not above 0.1 c.c. was added to any tube). In this series we used the *E. radicalis* (American and European strains and Clinton's *E. gyrosa* from *Quercus alba*) and *E. parasitica*. The *E. radicalis* from the oak (from Clinton's culture) is by nature a slower grower than the others, so that its incubation period was somewhat longer. In all cases the checks grew more slowly than those subjected to ether, showing an advantage in favor

of the larger quantities. The method of growth was similar to that developed in other liquid media.

In the light of these results a further and more careful study seemed necessary, especially as the ether appeared to be a favorable factor in the growth of the fungi. In this series the nutrient solution was measured, 10 c.c. being placed in each tube and 0.1 c.c. and 0.4 c.c. of ether being added. *Endothia parasitica* and *E. radicalis* (*E. virginiana*), the strains from Europe and from *Quercus alba* being used. The results confirmed those of the earlier experiment.

A more extensive series of cultures was next proposed. To 10 c.c. of culture solution ether was added in quantities of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 per cent., respectively. In this experiment only one species was used, *E. parasitica*. On the third day the check showed a good normal growth, that containing 0.05 per cent. ether was somewhat accelerated and that containing 0.1 per cent. was noticeably so. The next two members of the series showed slight germination, while those having 0.4 c.c. or over showed no apparent germination. The next day but little change was noticeable except that germination was apparent up to and including the tubes containing 0.6 per cent. ether. The second day later the culture containing 0.3, 0.4 and 0.5 per cent. ether showed accelerated growth while the one with 0.6 per cent. showed good growth.

On the tenth day after inoculation, the cultures with the higher percentages of ether showed signs of more vigorous growth, except the two highest members of the series, which never germinated. From this time on the results do not agree in all details with the earlier stages of the experiment, because of the unavoidable escape of varying amounts of ether from the different tubes and the consequent change of percentages. It was therefore not practicable to keep these cultures containing ether under observation for more than ten days or two weeks, a time too short for pycnidial formation in liquid media.

These results are represented in the form of a table below in which the check is rated 5 and the acceleration or retardation computed on this basis.

GROWTH OF ENDOTHIA PARASITICA IN LIQUID MEDIA TO WHICH THE INDICATED AMOUNTS OF ETHER WERE ADDED. (AMOUNT IN C.C.)

Days After Inoculation.	0.0	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
3	5	7	9	2	1	0	0	0	0	0	0	0
4	5	7	9	4	3	2	1	2	0	0	0	0
5	5	5	6	5	4	4	3	3	0	0	0	0
6	5	5	6	5	5	5	4	3	0	0	0	0
7	5	6	7	6	5	5	4	3	1	0	0	0
8	5	6	6	6	5	5	4	3	2	1	0	0
10	5	7	6	6	5	5	4	3	2	3	0	0

It appears that small quantities of ether have a stimulating effect on the fungus, quantities of from 0.2 per cent. up retard germination, that quantities from 0.4 per cent. up have injurious effect on the growth of the fungus. About this time we were informed that the extracts to be used could be supplied dry, therefore this line of work was not carried further. While it is possible to grow the mycelium under the influence of ether, the volatile nature of this chemical makes it impossible to keep such cultures intact long enough for pycnospores to appear. After two to two and one-half weeks the mycelium showed signs of dying and pulling away from the glass in the cultures containing the higher percentages of ether.

To Test the Effect of Tannin on Germination.

Two series of experiments were made with varying strength of aqueous solutions of these materials. They were conducted to test the power of the fungi to live in pure solutions of tannin and related substances. In the first series, the spores were sown on No. II agar and after germination cubes about 5 mm. each way were transferred to the solutions. In the second the spores were sown direct. In these tests *E. parasitica* and the European strain of *E. radicalis* were used.

The first test included Merck's tannin, and Kerr's extracts "A" and "1-X," of 2.05 per cent. and 5.0 per cent. solution. The strength of the solution appeared to have less effect on the fungi than did the nature of the material. Moreover, the two species of fungi showed no more difference in quantity of mycelium and vigor of growth than would be expected for two strains of the same species.

On tannin neither species grew even as much as might have been expected from the nutriment stored in the agar block.

On extract "A" a fair growth was made, using up the food material stored in the original agar block and forming masses of mycelium about 2 cm. across and producing abundant pycnospores.

On extract "1-X" an abundant growth was secured, entirely filling the liquid in the flask with a dense mycelium which rose above the liquid and produced abundant pycnospores.

The second series was made up in strength of 0.2, 0.4, 0.6, 0.8 and 1.0 per cent., respectively, of tannic and gallic acid. These were sown with spores of the same fungi used before. In no case was growth made although the checks showed the spores to be viable.

III.

ENDOTHIA ON TANNIN (MERCK).

Growth of Endothia on Commercial Tannin (Merck).

The cultures used for this series were made to contain 0.1 per cent., 0.2 per cent., 0.4 per cent., and by intervals of 0.2 per cent., up to 2.4 per cent. of tannin. In this series of experiments the agar remained firm, except in one or two of the cultures containing the highest percentages of tannin in which there was a slight tendency to a semi-fluid condition. No culture showed sufficient proteid digestion to permit the formation of a liquid. The following strains of *Endothia* were used. *Endothia parasitica* (Stevens No. 1158 and Clinton 7672 and 7675), a Chinese strain from the United States Bureau of Plant Industry,[1] and a strain designated P. P. of our own isolation from Prospect Park (Brooklyn) material;[2] *E. radicalis* (Metcalf No. A, Stevens No. 2391), and a European strain secured from Anderson and Clinton's *E. gyrosa* (No. 7674 and 7677); and *E. radicalis mississippiensis* (Stevens Nos. 1196, 2424 and 2443).

[1] This culture was made from material sent directly from China to the United States Bureau of Plant Industry.

[2] This culture was from the last living tree in Brooklyn (standing on the property of T. J. Sinnott, 1 Parkside Court). It was a Spanish chestnut 119 years old and was felled early in 1912. It was about 60 feet in height and over 3 feet girth. Analysis by Mr. Kew showed the exceptionally high tannin contents of 13 per cent.

Endothia parasitica.

The American strain of this species showed good growth, from the first producing a well defined pellicle over the surface of the cultures and producing a good aerial growth. This last varied in amount in direct ratio to the percentage of tannin present. By the end of the first week yellow pigment was beginning to appear in the mycelium. Aerial growth did not appear in abundance on the check until the end of the second week. Discoloration of the agar began during the second week. The subsequent bleaching began in the third week. Cultures from this series originally containing .2 gm. tannin (2 per cent.) were sent to Mr. Kerr for analysis, who reported that he was unable to detect any positive trace of tannin, but that he found a trace of gallic acid not exceeding .002 gm. This appears to indicate that the fungus can use the tannin as food.

At the end of six weeks pycnospores began to appear on the check. A week later the cultures containing 0.1 per cent. of tannin began to produce pycnospores. By the eighth week pycnospores had appeared on cultures containing as much as 1.4 per cent. of tannin. Ultimately, pycnospores developed throughout the entire series. The aerial growth was of a grayish hue in the cultures containing the higher percentages of tannin. So far as the inhibiting influence of tannin is concerned the two strains showed very little difference.

The Chinese strain grew well on this medium, showing about the same character as did the American strains, except that aerial mycelium appeared from three to five days earlier and was not so gray as was that of the American strains of this species. Pycnospores began to appear during the fifth week on cultures containing 0.4 per cent., 0.6 per cent., and 2.4 per cent. of tannin. Pycnospores ultimately appeared throughout the series of cultures. The Chinese strain was less affected by tannin than were either of the American strains of the species. However, the results do not tend to show any deleterious effects of tannin (Merck) on this fungus. It is very remarkable that this Chinese strain is more tolerant than the American strain, and raises the question as to whether this resistance is due to origin, age, or to modification of the fungus since its first introduction into America or to some other cause.

Endothia radicalis.

The American strains of this species showed a good growth forming a pronounced pellicle at first and abundant aerial mycelium later. Yellow pigmentation of the mycelium appeared during the first week. In some of the cultures this pigmentation extended to the aerial mycelium. However, in the cultures containing the higher percentages of tannin, the aerial mycelium was ashen. The agar in all the tubes oxidized, turned black, and did not again become lighter. During the fifth week, a few tubes began to exude a pinkish liquid. The addition of 0.2 per cent. of tannin to the medium acted as a stimulant, but in cultures containing more than 0.8 per cent. of tannin there was a marked retardation of growth. No pycnospores were produced. The strain from Dr. Clinton (labelled *E. gyrosa* No. 7677) showed some anomalies in its pigmentation, but otherwise agreed well with the other American strains of the species. In this strain, by the middle of the second week, the mycelium was highly pinkish, with the agar darkened. After a time the mycelium changed through gray to a yellowish tint.

The European strain of this species showed a greater resistance to tannin than did the American strains. While the growth and pigmentation was similar in the main to that shown by the American strains, the aerial mycelium was more healthy in color. Pycnospores were produced in fair abundance on cultures containing up to 1.2 per cent. of tannin after the eighth week. Tannin (Merck) has a decided inhibiting effect on both the American and the European strains, but more especially on the American.

Endothia radicalis mississippiensis.

The two strains of this fungus showed certain well defined differences throughout the series. No. 2443 grew more vigorously, producing more aerial mycelium, while No. 1196 showed deeper yellow pigmentation of the mycelium.

Growth was good from the first in these cultures, pigmentation beginning the first week. The agar was oxidized and later cleared only slightly. As compared with the check, the fungus appeared to have been but slightly affected by the presence of tannin (Merck)

either in growth or pigmentation. Neither does it appear that the fungus was able to use the tannin as food. No pycnospores were produced in either series of tubes.

SUMMARY.

From this series of cultures it appears that tannin (Merck) affects various species of the genus *Endothia* quite differently. *E. parasitica* may for a time be retarded in its growth, but subsequently it feeds on the tannin, using the entire supply in the cultures tested. At the other extreme is *E. radicalis mississippiensis*, which appears to be entirely unaffected by tannin, nor does it feed upon this substance. The cultures labeled *E. radicalis* (and Clinton's *E. gyrosa* No. 7677) are inhibited by the action of tannin. This is true of the American strains to a greater extent than of the European.

Growth of Endothia on Tannin (Merck). Second Series.

The culture media of this series were prepared with a new supply of Merck's tannin which had a greater digestive effect on the proteids of the agar than did the first supply. The percentages used ranged by 0.2 per cent. intervals from 0.2 per cent. to 3.0 per cent. of tannin. Sowings were made with *Endothia parasitica* (Clinton No. 7675 and the Chinese strain); two strains of *E. radicalis* (one of them being Clinton's *E. gyrosa* No. 7674), and *mississippiensis* (Stevens No. 2424).

Endothia parasitica (American).

At the end of the first week the check showed a good growth with pronounced surface pellicle and little aerial mycelium. In the cultures containing from 0.2 per cent. to 0.6 per cent. of tannin there was a good normal growth, while the cultures containing 0.8 per cent. of tannin showed but slight growth. Within the next three days the cultures containing 1.0 per cent. and 1.2 per cent. of tannin showed slight areas of discoloration indicating germination of spores. Growth on the other cultures continued as before. The medium also showed signs of oxidation in the tubes having the

best growth of mycelium. By the beginning of the third week the aerial mycelium was beginning to turn gray. Growth was present in slight degrees up to the cultures containing 1.6 per cent. of tannin. By the middle of the third week growth had begun in the liquid in the cultures containing 1.8, 2.2, 2.4 and 2.8 per cent., respectively, of tannin. No growth ever appeared in the cultures containing 2.0, 2.6, or 3.0 per cent. of tannin. Some clear exudation was present on cultures containing 0.8 per cent. of tannin at the beginning of the fourth week, while on the lower cultures prepycnidial pustules were beginning to appear. At the end of the first month a few pycnospores were present on those containing 0.8 per cent. of tannin. The agar began to clear during the third week, indicating tannin consumption by the fungus. No pycnidia appeared on the check. Pycnidia appeared rather freely on the cultures containing 0.2 per cent. to 0.4 per cent. of tannin while on those containing from 0.6 per cent. to 1.0 per cent. they were abundant. At the end of the second month large masses of mycelium were present at the sides of the tubes above the liquid except in the one containing 2.8 per cent. of tannin.

It appears that tannin accelerates growth development and fructification of this species, provided the resultant medium is not sufficiently acid to produce proteid digestion sufficient to make the medium liquid to any considerable degree.

Endothia parasitica (Chinese).

The development of this series of cultures was quite similar to that of the American strain, but some points of variation were noted. At the end of the first week the check and the cultures containing up to 0.4 per cent. of tannin showed good normal aerial growth, while cultures containing up to 1.0 per cent. of tannin showed slight mycelium development. Three days later growth was appearing on the cultures containing 1.2 per cent. of tannin. The medium was oxidizing in the tubes where growth was taking place. By the end of the second week aerial growth of the fungus was quite abundant in cultures containing up to 0.4 per cent. of tannin. Yellowish pigment was also beginning to appear in these cultures. By the beginning of the third week the cultures containing 1.2 per cent. to 1.8 per cent. and 2.4 per cent. and 2.6 per cent. were showing some growth. The agar began to lighten in color at the end of the

third week. About the same time a slight growth appeared in the cultures which contained 2.0, 2.2 and 2.8 per cent., respectively, of tannin. No growth appeared in the cultures containing 3.0 per cent. of tannin. At the end of the second month no pycnidia had appeared. Amber colored exudations were present on the cultures containing from 0.4 per cent. to 1.2 per cent. of tannin. Considerable masses of mycelium appeared on the sides of the tube where the agar contained 1.8 per cent. or less of tannin, as well as on the tubes containing 2.4 per cent. In the cultures containing 2.0 per cent. and 2.6 per cent. or above of tannin but little growth occurred. The presence of tannin seems to retard germination, but to accelerate subsequent growth as in the American strain of the fungus. However, the Chinese strain of this species does not appear to have as great tolerance for tannin (Merck) as does the American.

Endothia radicalis (Clinton's E. Gyrosa No. 7674).

At the end of the first week the check and the cultures containing from 0.2 per cent. to 0.6 per cent. of tannin showed a good healthy growth of mycelium with a decided pellicle and considerable aerial growth. The cultures containing 0.8 per cent. and 1.0 per cent. of tannin showed a slight growth of mycelium. The culture containing 1.2 per cent. of tannin showed darkened spots in the agar indicating germination of the spores. The other cultures were sterile. This relative development continued for several days. At the beginning of the second week all the cultures mentioned above showed rapid growth, while mycelium was beginning to appear on the cultures containing 1.2 per cent. of tannin and evidences of germination were present in the cultures containing 1.8 per cent. and 2.0 per cent. of tannin. The agar in cultures containing below 0.8 per cent. was beginning to show darkening due to the oxidation of the tannin.

The end of the second week brought additional growth on all the cultures which showed mycelium at the beginning of the week as well as those containing 1.4 per cent. to 2.0 per cent. of tannin. Above this no growth was evident. On the cultures containing 0.2 per cent. of tannin the aerial mycelium showed zonation. This persisted on these cultures to the end of the time they were under observation.

At the beginning of the third week submerged growth had ap-

peared in the tubes containing liquefied media with 2.6 per cent. or less of tannin. By the middle of the third week growth was apparent in the entire series of tubes. The mycelium remained a healthy color with only a slight graying of the aerial growth. The agar became darker but never bleached perceptibly, showing that, while tannin was oxidized, it was probably not used to any great extent by the fungus.

By the end of the second month the cultures which contained liquefied media had produced large masses of mycelium on the sides of the tubes above the liquid, except in those tubes which contained 2.2 per cent. and 2.6 per cent. or more of tannin. Little pigmentation of the mycelium was developed and no pycnospores were produced. Growth was apparently retarded by the presence of tannin except in very small quantities.

Endothia radicalis mississippiensis.

This series of cultures failed to show any germination in cultures containing more than 0.8 per cent. of tannin. At the end of the first week only those cultures containing 0.2 per cent. of tannin showed germination. At the beginning of the second week germination had taken place in the cultures containing 0.6 per cent. and 0.8 per cent. of tannin, but in no case was the growth equal to that of the check. At the end of the second week germination was completed in that no additional cultures containing more than 0.8 per cent. of tannin showed germination. Pigmentation was evident in the check and cultures containing 0.2 per cent. of tannin. This was a rather deep red, later fading out to a dull sordid shade in the culture containing 0.4 per cent. of tannin and becoming slightly yellowish in the remaining tubes. The agar was also darkening but did not subsequently bleach to any appreciable extent. But little oxidation of mycelium occurred and no pycnidia were formed. It appears that this fungus has a very low tannin resistance.

SUMMARY.

The results obtained with this second series of cultures are practically the same as in the first series except that *E. radicalis mississippiensis* showed much less resistance to tannin in the second than in the first.

IV.

ENDOTHIA ON CHESTNUT EXTRACTS (KERR).

Growth of Endothia on Kerr's "1-X" Extract.

This material is described by Kerr as the water-soluble tannin of the chestnut bark. It is insoluble in alcohol and in similar solvents. It occurs in quantities of from 3 per cent. to 5 per cent. in the bark. The sample used was between 95 per cent. and 100 per cent. pure (page 7). The quantity available would not allow as extensive a series of cultures as were used for commercial tannin products. Accordingly, the percentages used were 1.0, 1.2, 1.6, 2.0 and 2.4. The agar remained firm in all cases. Inoculations were made with *E. parasitica* (Stevens No. 1158) and *Endothia radicalis gyrosa* (Clinton *E. gyrosa* No. 7674).

Endothia parasitica.

Endothia parasitica on agar containing the "1-X" extract formed a heavy pellicle followed by a more or less copious aerial growth. The higher percentages of the extract stimulated the growth of aerial mycelium, but it was not until the third week that copious aerial growth began to appear. Pycnospores began to appear about the fifth week. Pycnospores were produced most abundantly on cultures containing 2.0 per cent. of the extract. The number of pycnospores decreased with the decreased percentage of extract. However, but few pycnospores were formed on the cultures containing 2.4 per cent. of this extract.

It appears that the presence of this form of tannin in relatively normal and sub-normal quantities stimulates the growth of this fungus. Indeed, maximum pycnospore production was observed at the point in the series where the percentage of the extract approached most nearly to the normal bark content.

Endothia radicalis (Clinton's E. Gyrosa No. 7674).

A growth very similar to that of *E. parasitica* was obtained with this organism. The pellicle was less evident and the length of the aerial mycelium much greater. This aerial growth was more copious than is normal for this species on agar without the addition of "1-X" extract. At the end of the first week after inoculation the maximum aerial growth was on the cultures containing 2.0 per cent. of the extract, while the most nearly normal and healthiest appearing growth was on the cultures containing 1.2 per cent. of the extract. Two days later the mycelium was showing the characteristic yellow pigment on the cultures of the two lower percentages. In the course of another week all the cultures showed the yellow color. By the beginning of the third week all growth had ceased. The agar was beginning to show signs of drying. However, it was almost ten days before pycnospores began to appear. The cushion-like groups of mycelium which preceded pycnidial formation began to appear toward the beginning of the third week after inoculation. By the beginning of the fifth week a few pycnospores were present on all the cultures. While the growth was good throughout the series, it was more nearly normal in those cultures of the series which contained less than 1.6 per cent. of the extract, while the most abundant pycnospore production was on those cultures containing 1.2 per cent. of the extract.

On the whole, small quantities of this extract stimulated the production of pycnospores, while the higher percentages tended to the production of a more vigorous growth of aerial mycelium and a consequent lessening of the quantity of pycnospores.

SUMMARY.

From these two series of inoculation, it appears that extract "1-X" is beneficial to the fungi of the genus *Endothia* in so far as tested and that even saprophytic species are stimulated by its presence. In optimum quantities (sub-normal percentages from the standpoints of the host content) the growth is increased, while in super-optimum quantities (normal or super-normal percentages) growth of the mycelium is stimulated at the expense of pycnidial production. It is rather surprising that the extract which is so

nearly pure tannin is not toxic, while the commercial tannin is toxic to this fungus. The culture medium itself passed through a series of changes in color. After a few days' growth had been made by the fungus, the culture medium turned darker as if the extract were being oxidized. This was followed about the end of the period of growth by a bleaching of the culture medium. This suggests that the fungi were able to oxidize and use at least a part of the extract as food.

Growth of Endothia on Kerr's "2-X" Extract.

The extract designated as "2-X" is in all its reactions similar to that designated "1-X" except that it is soluble in both water and alcohol. Its effect on agar is quite different, however, showing a tendency to digest proteids as do acids and so render the medium viscous. A series of cultures was prepared containing 1.0, 1.2, 1.6, 2.0 and 2.4 per cent., respectively, of the extract and inoculated with *Endothia parasitica* (Stevens No. 1158) and *E. radicalis* (*E. gyrosa* Clinton No. 7674). The cultures of the two upper members of the series were quite noticeably viscous.

Endothia parasitica.

The cultures used for this series of inoculation showed a more complete digestion of the proteids than did those which were used for *E. gyrosa*. At the end of the first week, growth was about normal on these lower members of the series (1.0 per cent., 1.2 per cent. and 1.6 per cent.), while the germination had apparently not taken place on the two higher percentages (2.0 per cent. and 2.4 per cent). About a week later growth began to appear on the cultures containing 2.0 per cent. of the extract. After the lapse of over four weeks, growth of aerial mycelium appeared on the cultures which contained 2.4 per cent. of the extract. Later some of the cultures exuded a clear liquid from the surface of the colony. About the sixth week yellow pigment began to show in the mycelium of the upper part of the series, *i. e.*, on those cultures containing 1.6 per cent. or more of the extract. After the lapse of over two months, pycnospores were produced rather abundantly on the cultures containing 1.6 per cent., 2.0 per cent. and 2.4 per cent. of the extract.

Endothia radicalis (Clinton's E. Gyrosa No. 7674).

At the end of the first week there was a good growth of the fungus on the cultures containing up to 1.6 per cent. of the extract, while it was two or three days later before growth became apparent in the next number of the series (2.0 per cent.). At the beginning of the third week the appearance of this series of culture was changed, the growth of aerial mycelium being greater than on any of the other culture media used. On those cultures which contained 1.0 per cent. and 1.2 per cent. of the extract there was an abundant growth of the aerial mycelium. The quantity of this decreased as the highest percentage (2.4 per cent.) tubes were reached. Some of the cultures had begun to exude a small amount of clear liquid. In the softer media at the upper end of the series growth was chiefly sub-surface and on the sides of the glass. The aerial mycelium on this series of cultures was a gray instead of a pure white as in the checks. The color indicated the presence of tannin, but, as no tests were made, no positive statement can be given as to the cause of the discoloration. By the fifth week a few pre-pycnidial cushions were forming. However, it was almost three weeks before pycnospores appeared on any culture. The first were on those cultures containing 1.2 per cent. of the extract. Pycnidia were ultimately produced on the cultures containing 1.0 per cent. and 1.6 per cent. of the extract. The most copious pycnidial production being on the 1.6 per cent. culture.

SUMMARY.

The extract designated as "2-X" is not so favorable for the growth of the fungus as that designated "1-X." It has a tendency to discolor the mycelium and to retard both growth and fructification and to reduce the quantity of pycnospores produced. These results are also surprising when compared with the results obtained in growing the fungus on commercial tannin.

Second Series of Tests of the Endothia on Kerr's "2-X" Extract.

When the second series of tubes were made up a larger quantity of the extract was available so that the series included 0.2, 0.6, 0.8, 1.2, 1.6, 2.0, 2.2, and 2.4 per cent., respectively, of the extract. Three species of fungi were used. *Endothia parasitica* (Clinton No. 7672 and the strain from China), *E. radicalis* (*E. gyrosa* Clinton No. 7674) and *E. radicalis mississippiensis.*

Endothia parasitica (American).

At the end of one week good growth had appeared on cultures with up to 1.2 per cent. of the extract; two or three days later they showed a growth of the fungus which was quite typical throughout the series. The higher percentages of the extract retard germination of the spores, but later accelerate the development of aerial mycelium. Slight graying of the mycelium was noted, but the medium passed through the changes in color which indicated tannin digestion by the fungus. About the first of the sixth week, the cultures containing 2.4 per cent. of the extract began to show pycnospores. Finally, after the lapse of about four weeks more, there were abundant pycnospores on all the cultures which contained 1.6 per cent. or more of the extract designated "2-X."

Endothia parasitica (Chinese).

At the end of the first week the cultures containing 0.2 per cent. of the extract showed a healthy growth of mycelium. By the middle of the week yellow pigment was apparent in the mycelium on these cultures and growth was evident on the cultures containing 0.6 per cent. tannin. At the end of the second week the pigment was turning to a reddish color on the 0.2 per cent. cultures, while none had appeared on the other cultures. The mycelium was somewhat ashen as though discolored by the tannin, while the pigment turned from reddish yellow to yellow, the red being most pronounced on the cultures containing 0.2 per cent. of the extract and disappearing with the increase of tannin. At the end of two months pycnospores had appeared, though on most of the cultures the pre-

pycnidial cushions were evident. The growth was slightly greater, and the pigmentation more pronounced, than in the American form of the species.

Endothia radicalis mississippiensis.

The pycnospores used in this inoculation appeared to be very weak and but few of the cultures on either this or the other media produced a growth of mycelium. While it appeared that this fungus is highly susceptible to the effects of tannin extract "2-X," the data are insufficient to warrant a conclusion. In those cultures which grew there was a good aerial mycelium produced but there were no pycnospores.

Endothia radicalis (Clinton's E. Gyrosa 7674).

The results obtained from this series do not differ materially from those obtained from the first series of *E. gyrosa* except that the aerial growth was perhaps a little more copious than in the former series. No pycnospores were formed on any of the cultures up to the end of the third month.

SUMMARY.

The second series of cultures with the tannin extract designated as "2-X" did not differ materially from those of the first series. This extract has a tendency to discolor the aerial mycelium, giving it an ashen hue. Germination is retarded, even for several weeks. Aerial mycelium is frequently more abundant than on the check cultures to which no tannin has been added. There is also a tendency to dull the color pigment. Growth is retarded at first, but later accelerated, while pycnidial production was greatly reduced.

Growth of Endothia on Kerr's "3-X" Extract.

The extract designated "3-X" is the coloring matter of the bark. While this is estimated as tannin in bark analysis, its real nature is unknown. It precipitates gelatine and combines with hide, but does not give the same distinct reactions with metallic salts as do

other tannins. The sample used was between 85 and 90 per cent. pure. As the quantity available was very small it was used only in the proportions of 1 per cent. and 2 per cent. Both *Endothia radicalis* (*E. gyrosa* Clinton No. 7674) and *E. parasitica* (Stevens No. 1158) were grown on this medium.

Endothia parasitica (American).

At the end of the first week, there was only a slight growth apparent on the cultures containing 1 per cent. of the extract. This growth was in the form of a dense pellicle rather than aerial. It increased in quantity for about two weeks. By this time the aerial growth began to be pronounced, the mycelium following up the sides of the tubes rather copiously. The cultures containing 2 per cent. of the extract were retarded about ten days in germination, but never grew as well as did the fungus with only 1 per cent. of the extract. The growth on this medium was never normal, always having the appearance of being almost dead. During the first week a few pycnospores appeared on the cultures containing 1 per cent. of the extract. The cultures containing 2 per cent. of the extract never produced pycnospores.

Endothia radicalis (Clinton's E. Gyrosa 7674).

At the end of the first week there was a light aerial growth on the cultures which contained 1 per cent. of the extract. The cultures with 2 per cent. were retarded about a week in germination and did not grow so well as those on 1 per cent. The fungus had a tendency to form a thick brown pellicle and to form some aerial mycelium. At the end of the second month a few pycnospores appeared on the cultures with 1 per cent., but none were ever formed on those with 2 per cent. extract.

SUMMARY.

From the limited number of cultures available it appears that the extract designated "3-X" is very unfavorable to the growth of species of *Endothia*, and that its presence in large quantities in the tree would tend to check the growth of the fungus. This extract

gave the poorest growth of any of the extracts used in the first series of experiments, commercial tannin not excepted. The growth of the fungus was never normal and pycnidial production was very decidedly checked. These results are also surprising in that this extract, which is primarily coloring matter of the bark which, under ordinary methods of analysis, is estimated as "tannin," is more toxic than commercial tannin. Mr. Kerr in commenting on these results, says, "The action of '3-X' is also surprising, as it is what we term the coloring principle of the bark, the exact nature not having been determined by any one that I know of. Its action brings out a rather interesting point, and that is that chestnut trees of Northern growth, say on a line North of the Southern boundary of Pennsylvania, contain very materially less coloring matter than the growth South of it and, as we all know, the wood in the latitude referred to seems to have been more susceptible to the disease than that farther South." (Letter Dec. 26, 1913.)

Growth of Endothia on a Combination of Kerr's "1-X" and "3-X" Extracts.

Since "1-X," which is a tannin extract, was stimulating and "3-X," which is primarily coloring materials giving tannin reactions, was toxic, it was decided to combine the two into one extract. The material was made up into a series of cultures containing respectively 0.2, 0.6, 0.8, 1.2, 1.6, 1.8, 2.0, 2.2, 2.4, 2.6 and 2.8 per cent. Sowings were made with *Endothia radicalis* (*E. gyrosa* Clinton No. 7674), *E. radicalis mississippiensis* (Stevens No. 2424) and both American and Chinese strains of *E. parasitica.*

There was a tendency for the agar containing as much as 1.2 per cent. of the extract to become less solid but even with 2.8 per cent. there was no approach to a real liquid condition.

Endothia parasitica (American).

In this series the sowings on the cultures containing 0.2 per cent. and 2.2 per cent. failed to germinate. At the end of the first week there was a healthy appearing growth with considerable aerial mycelium on the cultures containing 0.6 per cent. and 0.8

per cent. of the extract, while there was a decreasingly smaller growth throughout the series. In the cultures containing the higher percentages of the extracts, this was very slight indeed. By the end of the second week the characteristic grayish color produced by tannin compounds appeared in the cultures of this series. The relative amount of growth throughout the series remained about the same, the lower number being most copious and covering the entire area of the agar surface, while in the higher per cents. the fungus has the tendency to grow in small clumps as though the mycelium formed from separate spores did not penetrate the agar sufficiently to form a continuous growth. By the middle of the third week a yellow pigment was becoming noticeable throughout the series, highest in the cultures with 0.6 per cent. of the extract and gradually fading out toward the end of the third week, pycnidia began to appear on the cultures containing 0.6 per cent. of the extract. By the end of the fifth week pycnospores had appeared on the cultures containing up to 2.2 per cent. of the extract. At the time observations ceased, at the end of the second month, pycnidial production had extended throughout the series. The pycnospores were produced most copiously on those cultures containing up to 2.0 per cent. of the extract. There was a marked decrease in pycnospores produced above this point. The growth was always of an unhealthy tint and did not show as good pigmentation as was to be expected in the species. There was only a very slight clearing of the medium. This condition indicates but little effect on the extract by the fungus, as compared with some other cultures.

Endothia parasitica (Chinese).

As before, no growth was observed on those cultures containing 0.2 per cent. and 2.2 per cent. of the extract. At the end of one week there was some growth on the cultures containing 0.6, 0.8 and 1.2 per cent. of the extract. By the middle of the second week the cultures containing 0.6 per cent. of the extract were covered with a grayish aerial mycelium less copious than normal. The cultures containing up to 2.8 per cent. of the extract showed a slight growth of mycelium, which was always in patches. By the first of the third week, on the higher percentages of the series, pigment was appearing in the pellicle on the surface of the tubes. This varied from rich yellow on 0.6 per cent. of the extract to cream

colored on 2.6 per cent. At the end of the first month no pycnospores were present. The medium was slightly clearer in color. At the end of two months pycnospores were still absent and the medium only slightly cleared. It appears from this that the Chinese strain of the fungus is more susceptible to the effects of this extract than is the American strain. As the check is also sterile the failure to produce pycnospores cannot be attributed to this extract.

Endothia radicalis (Clinton's E. Gyrosa 7674).

At the end of the first week there was a fair growth of mycelium of almost normal color on the cultures containing 0.2, 0.6 and 0.8 per cent. Above this throughout the series there was a slight growth which was less normal in appearance. By the end of the second week all the cultures showed yellow pigment varying in intensity, indirectly with the amount of the extract present in the medium. The yellow pigment was only fairly well developed and most of the aerial mycelium had a gray cast. At the end of the third week most of the cultures had the agar surface entirely covered. The majority of the cultures showed a very bright yellow pigment. No pycnospores appeared on any of the cultures. The extract does not appear to have been affected perceptibly by the growth of the fungus as it had bleached but little. It appears that this extract induces abnormally abundant yellow pigment in this species, retards its growth, and is not conducive to pycnidial formation, although the check is sterile.

Endothia radicalis mississippiensis.

This species did not prove a good subject for our work, but more inoculations with it were successful on this extract than on any other. The successful series included cultures containing 0.6, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 per cent., respectively, of the extract. At the end of the week only one culture of the series showed any growth. This contained 0.8 per cent. of the extract. The growth was good with considerable aerial mycelium. By the middle of the week cultures with 0.6 per cent. and 0.8 per cent. of the extract showed a good surface growth with some aerial mycelium. Fair growth was also present in cultures with 1.0 per cent. and 1.6 per cent. of the extract, and a slight growth in cultures with 2.0 per

cent. and 2.8 per cent. of the extract. By the end of the second week yellow pigment was beginning to appear in the cultures in abundance. Much aerial mycelium developed on all the cultures. By the end of the second month there was almost no bleaching of the medium, the pigment was normal, as was also the color of the aerial mycelium. Pycnospores were present in rather large numbers in the cultures containing up to 2.0 per cent. of the extract.

SUMMARY.

This extract appears to be utilized by two of the species of *Endothia*, namely, *E. parasitica* and *E. radicalis* (*E. gyrosa*). While these species do not thrive as well on a medium containing this extract as on agar from which it is absent, it does not seem to exert nearly so detrimental an influence on the American strain of *E. parasitica* as it does on the Chinese strain of this fungus. It appears to have less effect on *E. radicalis mississippiensis* than on the other species tested. On the whole, it appears to cause an increase of aerial mycelium of a grayish color, an increase of pigmentation and a decrease in fructification.

Growth of Endothia on Kerr's Extract "A."

Extract "A" is a compound of various forms of tannin and of other more or less related substances. It represents about 9 per cent. of the dry weight of the bark. Its composition is as follows:

60% tannin, embodying the forms represented as "1–X," "2–X" and "3–X."
10% fermentable sugars.
7% gallic acid.
8% pentoses and pentosans.
5% water.
10% undetermined.

This extract produces a rather advanced proteid digestion, causing the agar to become semi-fluid, and in the higher percentages used, a considerable amount of fluid was present. The series included 1.0, 1.2, 1.6, 2.0 and 2.4 per cent., respectively, of the extract. Sowings were made with *Endothia radicalis* (*E. gyrosa* Clinton No. 7674) and *E. parasitica* (Stevens No. 1158).

Endothia parasitica (American).

At the end of the first week, there was a slight growth of mycelium throughout the series of cultures with some evidence of a surface pellicle on those which contained 1.0 per cent. of the extract. By the middle of the second week, there was a good growth up to the cultures containing 2.0 per cent. of the extract. The growth in subsequent weeks was very slow, the aerial mycelium was ashen and altogether suggested Extract "3-X." At the end of the first month, pycnospores began to appear on the cultures containing 1.0 per cent. of the extract. No other pycnospores appeared. The fungus made its poorest growth on this medium.

Endothia radicalis (Clinton's E. Gyrosa 7674).

By the end of the first week, there was a slight growth of aerial mycelium on the culture, with a slight surface pellicle in those containing 1.0 per cent. of the extract. Growth continued to be poor and ashen in color throughout the series. At the end of the first month, growth became a little more rapid for a short time. No spores were produced, with the exception of one or two pycnidia in one of the cultures which contained only 1.0 per cent. of the extract.

SUMMARY.

Of the four extracts furnished by Kerr, the one designated "A" was the most toxic, probably because of the presence of the material designated "3-X." Growth of the fungus was scant, the mycelium was never healthy in color and pycnospores were formed very sparingly.

V.

GROWTH OF ENDOTHIA ON "TANNIN COMPOUND."

The substance designated as "tannin compound" was made up according to the formula of Kerr's extract "A" from the materials at hand.

60 gm. tannin (Merck).
10 gm. dextrose.
7 gm. gallic acid.
8 gm. arabnose.

85 gm. total.

This material was added to the medium in the same manner as was the tannin. The series of cultures prepared were at intervals of 0.2 per cent. from 0.2 per cent. to 3.0 per cent. The effect on the proteids was the same for this substance as for tannin. The higher percentages digested almost all proteids so that the medium was a clear liquid. Sowings were made with *Endothia radicalis mississippiensis* (Stevens No. 2424), *E. radicalis* (*E. gyrosa* Clinton No. 7674), *E. parasitica* (American Clinton No. 7675) and Chinese.

Endothia parasitica (American).

At the end of the first week a good growth of mycelium, almost normal and covering at least half the surface of the agar, was present in the cultures containing up to 1.0 per cent. of the compound. Fair growth occurred in more restricted area on the cultures up to 1.4 per cent. of the compound, while a very slight discolored area on one of the cultures containing 1.8 per cent. of the compound indicated the germination of the spores. From 0.6 per cent. up there was a slight graying of the mycelium.

By the middle of the second week, growth was more copious, covering the entire surface of the agar in the cultures containing 0.8 per cent. or less of the compound, in a few cultures showing more surface pellicle and zonation of aerial mycelium; growth was fair up to the cultures containing 1.6 per cent. of the compound. Oxidation of the substances in the agar was apparent up to 1.0 per cent. of the compound. This was shown by the darkening of the agar. By the end of the second week the aerial mycelium was

noticeably ashen in color in those cultures where growth was most abundant. Darkening of the agar indicative of spore germination was noticeable on the series up to those cultures which contained 2.2 per cent. of the compound. During the third week a slight mycelial growth appeared on the cultures containing 2.4 per cent. of the compound.

At the end of the third week the cultures in which the agar remained solid (*i. e.*, up to 2.0 per cent. of the compound) showed a good growth of mycelium. Zonation had practically disappeared and the agar was beginning to take on a lighter color. In those cultures which contained more or less liquid, there was at least a slight submerged growth even in the cultures which contained 3.0 per cent. of the compound. During the fourth week an amber colored exudation appeared on the cultures containing 0.2 per cent. of the compound. Within the next week this exudation appeared on the cultures containing 1.2 per cent. or less of the extract. Pycnospores appeared on the cultures containing 1.0 per cent. or less of the compound. Aerial growth on the solid cultures was completed but the submerged growth on the liquid cultures increased rapidly.

At the end of the second month, pycnospores were present on the cultures containing 2.2 per cent. or less of the compound. Abundant conidial production was confined to those cultures which contained from 0.6 per cent. to 0.2 per cent. of the compound. Large patches of mycelium were formed on the sides of the tubes throughout the series of cultures with a liquid medium. This compound did not simulate the effect of Extract "A" as closely as it did that of Merck's tannin.

Endothia parasitica (Chinese).

At the end of the first week there was a good normal growth on the cultures containing up to 0.8 per cent. of the compound and a less abundant growth up to the cultures containing 1.6 per cent. An olive spot on one of the 1.8 per cent. cultures indicated germination. This resembled quite closely the American race of this species on the same medium. At the end of the second week, there was a copious growth on the cultures containing up to 1.0 per cent. of the compound with less growth on the cultures containing up to 1.6 per cent. of the compound. Dark spots indicated germination up to 2.2 per cent. cultures. The medium was darkening in the

lower part of the series, because of oxidation of the tannin. By the beginning of the third week submerged growth was beginning to appear in most of the liquid cultures, and the aerial mycelium of the solid cultures becoming gray.

No growth ever appeared in the cultures containing 3.0 per cent. of the compound. In all the liquid cultures, except that containing 3.0 per cent. of the compound, large clumps of mycelium were formed on the sides of the tube. At the end of the second week, there was an amber colored exudation on several of the cultures containing from 0.4 per cent. to 0.8 per cent. of the extract. No pycnospores were formed. This strain of *Endothia parasitica* appeared to be retarded more than does the American strain by the use of this compound.

Endothia radicalis (Clinton's E. Gyrosa 7674).

At the end of the first week there was a good healthy growth covering about one-half of the area of the agar in the cultures containing 0.2 per cent. and 0.4 per cent. of the compound. There was a fair growth on the cultures containing 0.6 per cent. and 0.8 per cent. of the compound, while blackened areas indicating germination were occurring in the cultures containing up to 1.8 per cent. By the middle of the second week good growth was apparent up to the cultures containing 1.6 per cent. of the compound. Slight growth was present in the cultures containing 1.8 per cent. and 2.2 per cent. of the compound. Oxidation of the medium was apparent on some of the cultures. Little relative change was noticeable before the beginning of the third week. The aerial mycelium on a few cultures was turning ashen, especially on those cultures containing from 0.6 per cent. to 1.0 per cent. of the compound. A slight submerged growth was also apparent through the liquid portion of the series.

By the end of the second month no blackening of the agar indicating tannin absorption had appeared. A very few pycnospores were formed on the cultures containing 1.0 per cent. of the compound. Large clumps of mycelium were formed on the sides of the tubes throughout the liquid members of the series. It appears that this species of *Endothia* is more susceptible to the detrimental effects of this compound than even the Chinese strain of *E. parasitica.*

Endothia radicalis mississippiensis.

At the end of the first week there was a good growth of aerial mycelium on the cultures containing 0.2 per cent. of the compound with slight growth on those containing 0.4, 0.6 and 1.0 per cent., respectively. No later growth appeared on the other cultures of this series. The growth was rapid, and the aerial mycelium was copious, reaching its development by the middle of the second week. The cultures developed a very copious bright reddish pigment, which faded perceptibly in the two upper members of the series. The agar was much oxidized but not discolored by the absorption of tannin. No pycnospores were formed.

SUMMARY.

This compound was made to test the possibility of preparing from commercial sources a compound similar in its effects to the extract designated by Kerr as "A." Such an expectation failed of realization, as might have been foreseen. In all cases except the American strain of *Endothia parasitica* pycnidial formation was greatly retarded. Growth was sufficiently similar to that obtained with Merck's tannin to need but little discussion here.

VI.

DISCUSSION OF RESULTS OF CULTURES.

In the paragraphs which deal with the experimental work on the relationship of species of *Endothia* to the various extracts used, the results have been grouped according to the materials employed in the culture medium. In order to better understand the relationship of these results, it is necessary to view them also from the standpoint of the various fungi used. These results are accordingly summarized under each of the species used in our experients.

Endothia parasitica (American and Chinese).

For the various cultures three strains of this fungus were used from American sources (Stevens No. 1158, Clinton No. 7672, and one of our own isolation designated P. P.) and the one from China. The culture from Stevens was used in the preliminary work while the one from Clinton was used in the later experiments. The results, however, agree for these two strains whenever used on the same medium. The strain designated P. P. was secured from a tree in Prospect Park, Brooklyn, which contained an excessive amount of tannin (page 17). It was hoped that this strain would show a greater tannin resistance than did the others, but such was not the case, at least to a perceptible extent.

On Extract "1-X" it appears that normal and subnormal quantities of this extract stimulate the growth of the fungus. Indeed, the maximum pycnidial formation and the normal percentage on this extract coincide in the cultures. In other words, this form of tannin is beneficial to the fungus, which probably uses it as a food. The extract designated "2-X" is also favorable to the development of the fungus. The extract designated "3-X" proved quite injurious to this fungus, causing a marked retardation in germination and a pronounced discoloration of the mycelium in the cultures containing the higher percentages. The combination of the "1-X" and "3-X" extracts gave a poor growth of mycelium which appeared unhealthy. Pycnidial production was below normal and was inhibited by the higher percentages (2 per cent. or more) of the extract. The extract designated "A" gave even poorer results than did the combination of "1-X" and "3-X." Growth was very poor and no pycnospores were produced. It appears that the tannin designated "2-X" serves as food for the fungus, while the coloring matter, which has some of the properties of tannin, acts as a check upon the growth of the fungus.

The tannin compound which was prepared after the formula of Extract "A" gave results quite similar to those obtained from commercial tannin (Merck) which formed a considerable part of its bulk. The areas of maximum pycnidial production, 1.2 per cent. of the compound, is considerably below that of the chestnut tannins.

On commercial tannin (Merck) the fungus grew well and normally with less aerial mycelium. The color was good except in

the cultures containing a high (2.0 per cent. or more) percentage of tannin. Tannin appears to accelerate the development of the fungus which is able to utilize it as food.

The results with the Chinese strains of this species were rather surprising. On the extract designated "2-X," growth was slightly more vigorous than that of the American strains of the species. Pigmentation of the mycelium was also brighter in the Chinese fungus. These characters appear to be abnormal, as no conidia were produced in the cultures.

On the combination of "1-X" and "3-X" extracts, the growth was poorer in the Chinese strains than in the American strain. The Chinese fungus also showed a slight tendency to utilize the tannin extract as food.

On the tannin compound the Chinese strain of the fungus made its poorest growth. The aerial mycelium was grayish. The Chinese fungus did not develop so well on this compound as did the American.

On commercial tannin (Merck) the Chinese strain of the fungus grew well, germinating earlier and producing a healthier colored mycelium than did the American strain of this species. It appears that the Chinese strain of the species has less tolerance for tannin than does the American.

Endothia radicalis.

This fungus was used in only one series of experiments. Three strains were employed, two from America (Metcalf No. A and Stevens No. 2443) and one from Europe (Anderson). These were grown on tannin (Merck) only. The paucity of pycnospores in cultures made these strains especially unsuited to extensive experimental cultures. In the American strains growth was good, with an abundance of yellow pigmentation of the mycelium. On those cultures containing the larger amounts of tannin the aerial hyphae were ashen in color. The fungus showed no tendency to use a considerable amount of the tannin from the agar. No pycnospores were produced. The European strain of this fungus showed a greater tannin tolerance than did the American strain. The aerial hyphae retained their normal color and pycnospores were produced abundantly on cultures containing as much as 1.2 per cent. of tannin. The tannin (Merck) has a decided inhibiting effect on both the American and European strains, but more especially on the American.

Endothia radicalis (Clinton's E. Gyrosa 7674).

This fungus grew well in the cultures and showed marked response to the various media employed. On the extract designated "1-X" there was on the cultures containing the higher percentages of the extract a super-normal growth of aerial mycelium. The maximum growth was reached in about seven days. The presence of 1.6 per cent. or more of the extract in the agar caused a less healthy growth than appeared on the cultures containing a lower percentage of the extract. Pycnospores began to form during the fifth week. Low per cents. of this extract stimulated pycnidial production, the maximum occurring on the cultures containing 1.2 per cent. of the extract, while higher percentages tended to induce a super-abundance of aerial mycelium and a proportionate lessening of pycnidial production.

The growth of this fungus on Extract "2-X" was quite similar in general to that described on "1-X." There was a slight exudation of a clear liquid from some of the cultures in this series. The aerial mycelium on the cultures containing "2-X" extract had a tendency to become gray instead of remaining white. Otherwise, the results from these media were very similar.

On the extract "3-X" germination was slightly retarded and growth less rapid than on the other two extracts. Pycnospores were produced on cultures containing 1 per cent. of the extract, but not on the cultures containing 2 per cent.

When the extracts designated "1-X" and "3-X" were combined growth was less healthy than on "1-X" alone and more robust than on "3-X" alone. That is, the growth was somewhat between that obtained on the separate extracts. The fungus on this medium produced no pycnospores. The pigmentation of the mycelium was abnormally abundant, growth was retarded and fructification delayed or inhibited.

Extract "A" gave very poor growth for this species with pycnidial production almost suppressed. The mycelium produced was decidedly ashen in color, showing a decided influence from the extract in the agar. The tannin compound which was made up to simulate Extract "A" gave good healthy growth of this fungus up to a strength of 1.6 per cent. of the compound. Above this the growth was poor. The aerial mycelium was quite gray. The fungus in time began to absorb the tannin from the agar. Scant pyc-

nidial production occurred in cultures containing 1.0 per cent. or less of the compound. This compound had a decidedly adverse influence on the fungus.

On commercial tannin (Merck) this fungus grew well at first. The first pigment formed in the mycelium was slightly pinkish, changing to gray and later succeeded by a yellow pigment. No pycnospores were produced. The agar never bleached, so that it is improbable that this fungus was able to utilize the tannin. This tannin may be said to have an inhibiting influence on the fungus.

It appears, then, that small quantities (under 1.6 per cent.) of the extracts designated "1-X" and "2-X" have a stimulating effect on the fungus at least for a time, while higher percentages of these extracts have an adverse influence on the fungus. All the other extracts used tended to inhibit the growth of the fungus and to prevent or reduce pycnidial production.

Endothia radicalis mississippiensis.

Two strains of this fungus were used in the course of our experiments. These were Stevens No. 2424, which was used throughout the experiment, and Stevens No. 1196, which was used but once. The extracts used were "1-X", "2-X" and "3-X," tannin compound and tannin (Merck).

This fungus showed a very marked susceptibility to tannin extracts, usually being entirely eliminated from the cultures by a comparatively low percentage of the extracts. On agar containing "2-X" extract no germination occurred on tubes containing 1.0 per cent. or more of the extract. On tubes containing 0.8 per cent. or less of the extract the fungus made a good aerial growth of mycelium, but produced no pycnospores.

The sowing on agar containing tannin compound showed germination where 1.0 per cent. or less of the extract was present. The aerial mycelium was copious and contained a pinkish pigment, fading out in the upper part of the series of cultures. The tannin, apparently, was not utilized and no pycnospores were formed.

On tannin (Merck) there appeared to be very little modification of growth. In one series of cultures growth occurred on media containing as high at 2.4 per cent. of tannin, while with tannin from another supply the fungus was eliminated by 1.0 per cent.

or more of tannin. No pycnospores were produced. The fungus did not appear to use the tannin for food.

On all these substances it appears that the fungus is eliminated, at least under certain circumstance, by a comparatively low percentage of tannin. In marked contrast is the behavior of the fungus on the extract designated "1-X" and "3-X." Here growth occurred on cultures containing as much as 2.8 per cent. of the extract. The growth throughout the series was in the main good and well pigmented. The fungus does not appear either to be affected by the presence in considerable quantities of this substance or to be able to utilize it as food.

VII.

GENERAL SUMMARY.

1. Results obtained with commercial tannin are not always comparable to each other or to those obtained from specially prepared extracts because of variations in chemical composition and the presence of tannin-like substances other than tannic acid. Commercial tannins of the same brand differ in their behavior in culture media as indicated by the growth of the various species of *Endothia* used in these experiments.

2. Commercial tannin and tannin in the plant are not the same. No extract will be the same as the substances in the plant.

3. Tannin is an anhydrous glucoside of gallic acid and is easily converted by hydrolosis into gallic acid and related substances. It is very doubtful if any culture medium can be prepared containing as much pure, unchanged tannin as was put into it. Therefore, we cannot know the exact percentage of tannin in a culture medium but we can put known amounts into those with which we are working.

4. The quantity and form of tannin compounds present in the substratum each exert an influence on the growth of the fungus. When the fungus attacks a plant we have no way of knowing the form of the tannin with which it comes in contact. However, it is quite evident that the tannin of the plant is associated with coloring materials and other substances, some of which are toxic. Furthermore, the fungus may be selective in its nutrition during either a part of or its entire existence and send its mycelium into certain tissues containing little or no tannin. The *Endothia parasitica* is

especially destructive because it works in the cambium cells, but later in life it pushes through the outer tannin-bearing cells of the bark for the production of its spores.

5. The character of the food supply influences the vigor of the fungus and, therefore, its power to resist the toxicity of the tannin and other materials with which it comes in contact. The amount and character both of the food supply and of the tannin and other materials no doubt vary with the seasons and the growing periods of the host plant.

6. In almost every instance, without regard to the form of tannin used or the fungus grown, a high percentage (0.8 per cent. or more) of tannin caused a retardation of germination, frequently followed by an abnormal stimulation to growth of aerial mycelium.

7. Species of the genus *Endothia* show a marked response to the presence of tannin and related substances in the culture medium.

8. The species of *Endothia,* and to a certain extent strains of the same species, show a considerable variation in their response to tannin and other substances. (*a*) *E. radicalis mississippiensis* was unaffected by the tannin, but did not use it for food and did not produce pycnospores in cultures containing tannin. (*b*) *E. parasitica* was slightly retarded in its germination and early growth, but later was able to use as much as 2 per cent. tannin as food. It was the only species studied that was able to utilize any considerable amount of tannin for food. *The American strains were more resistant to tannin and associated toxic materials than the Chinese strains.* (*c*) *E. radicalis* (including *E. gyrosa*) was very susceptible to the influences of tannin.

9. Tannin (Merck) affects the various species of *Endothia* very differently. *E. radicalis* (and *E. gyrosa*) are inhibited; *E. parasitica* is at first retarded and later is able to feed on the tannin; *E. radicalis mississippiensis* is practically unaffected by and does not feed upon tannin.

10. Tannin was utilized for food by *E. parasitica,* which is able to remove as much as 2.0 per cent. of tannin from the substratum.

11. There was no evidence that the other species of *Endothia* employed are able to use any considerable amount of tannin as food.

12. Analyses of chestnut bark made by Kerr show a corresponding tannin reduction in diseased areas which confirms the culture experiments and makes it possible to state that *E. parasitica* is able to use the tannic acid for food.

13. *Endothia parasitica* appeared to have its power of pycnidial production stimulated by commercial tannin and the true tannin extracts of chestnut bark, but to have its pycnidial production reduced or inhibited by those extracts of chestnut bark which are composed almost entirely of coloring substances but which are present in tannin extracts and estimated as tannin.

14. Specially prepared extracts of pure tannin were either stimulating or only slightly toxic when combined with coloring materials and other substances associated with tannins and responding in the same or in a similar manner to tannins.

(*a*) Kerr's "1-X" extract has a stimulating effect on *E. radicalis* (*E. gyrosa*) and *E. parasitica.*

(*b*) Kerr's "2-X" extract has a tendency to retard *E. radicalis* (*E. gyrosa*), *E. parasitica* (both American and Chinese strains) and *E. radicalis mississippiensis.*

(*c*) Kerr's "3-X" extract was extremely toxic to *E. radicalis* (*E. gyrosa*) and *E. parasitica.*

(*d*) In *Endothia radicalis* (*E. gyrosa*) conidial production was at its maximum at 1.0 per cent. to 1.2 per cent. of the extracts designated "1-X," "2-X" and "3-X," while but few, if any, pycnospores were produced on the other substances used.

(*e*) The European strain of *E. radicalis* showed similar results, but the American strain showed a tendency to remain sterile.

(*f*) A combination of "1-X" and "3-X" is somewhat toxic, but the toxicity of "3-X" appears to be largely overcome by the stimulating influence of "1-X." *E. radicalis* (*E. gyrosa*) and *E. parasitica* were slightly retarded and *E. radicalis mississippiensis* was very slightly retarded. The Chinese strain of *E. parasitica* was less resistant than the American strain.

(*g*) Kerr's "A" was most toxic of all compounds on *E. radicalis* (*E. gyrosa*) and *E. parasitica.*

(*h*) Tannin compound gave results similar to Merck's tannin instead of "A."

15. A supernormal growth of aerial mycelium was usually accompanied by a corresponding reduction in pycnidial formation.

16. Harmful effects of tannin were also frequently shown by the absence of natural pigment from the mycelial pellicle and by the ashen color of the aerial hyphae. One or both of these might be present in the same series of culture.

17. While tannic acid is no doubt toxic to many parasitic fungi, there are other substances associated with tannin which are also toxic. Some of these substances respond to the ordinary tannin tests and have probably been mistaken for tannin. The factors which enable plants to resist the attacks of parasitic organisms present an extremely complicated problem. The solution of this problem lies in the study of the chemistry and physiology of the cell.

18. Throughout the summary the terms "tannin" and "tannic acid" have been used in the generally accepted sense, but experiments with Kerr's extracts "1-X," "2-X" and "3-X" indicate that the toxic property is in the coloring material, which in analytical work is usually estimated as tannin.